Adv. Hort. Sci., 2022 36(3): 163-174 DOI: 10.36253/ahsc-13044

AHS
Advances in Horticultural Science

Secondary metabolite changes in Maymars juniper cuttings (*Juniperus sabina*) under different treatments of propagation (IBA, substrate and harvest time of cutting)

M. Abshahi [1], H. Zarei [2 (*)], B. Zahedi [1], F.A. García-Morote [3], A. Rezaei Nejad [1]

[1] *Department of Horticulture Sciences, Faculty of Agriculture, Lorestan University, Khorram abad, Iran.*

[2] *Department of Horticulture Sciences, Collage of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.*

[3] *Department of Agroforestry Technology and Science and Genetics, Higher Technical School of Agricultural and Forest Engineering, University of Castilla-La Mancha, Albacete, Spain.*

OPEN ACCESS

(*) **Corresponding author:**
h.zarei@gau.ac.ir

Citation: ABSHAHI M., ZAREI H., ZAHEDI B., GARCÍA-MOROTE F.A., REZAEI NEJAD A., 2022 - *Secondary metabolite changes in Maymars juniper cuttings (Juniperus sabina) under different treatments of propagation (IBA, substrate and harvest time of cutting).* - Adv. Hort. Sci., 36(3): 163-174.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 21 April 2022
Accepted for publication 7 July 2022

Key words: Antioxidant, flavonoid, IBA, phenol, rooting.

Abstract: The endemic Juniper of Maymars (*Juniperus sabina*) is one of the most valuable plants in forested areas. The objectives of this experiment were: I) to determine the best conditions for stem cutting propagation of this species, and II) to examine changes in some of the secondary metabolites during the four months (the first of each season): January, April, July, and October, after rooting of cuttings. The research was done with the treatment of five levels of Indole Butyric Acid, including: 0, 1000, 2000, 4000, and 8000 ppm in four rooting substrates, including perlite, perlite-cocopeat (1:1), pumice, and a mixed rooting substrate (sand, perlite, cocopeat, vermicompost, and potash; 1:1:1:1:1) in the four seasons of the year, with stem cuttings having an average length of 15 cm. The best treatment with more than 50% rooting was seen in April at levels of 4000 and 1000 ppm, and the best substrate was perlite cocopeat. Using lower levels of IBA led to a reduction in total phenol content in the cuttings during the rooting period. The flavonoid content of the cuttings varied across different seasons. Based on these results, we recommend this way of propagation for *Juniperus sabina* production. This propagation method takes less time in comparison with sexual propagation from seed.

1. Introduction

The genus *Juniperus* is one of the few conifers that act as a main tree in the natural ecosystems of the mountainous forests of the world. The

protective and valuable roles of various species of junipers in the management of forest erosion and water management are well known. Also, the role of junipers is important both in water storage and in soil conservation (Ali Ahmad Koruri *et al.,* 2011). They are great landscaping and ground cover species (Westerfield, 2012). Among the junipers, *Juniperus sabina*-Maymars is one of the most popular types of junipers. This species can be utilized for forest restoration on poor sites with low potential productivity, such as arid and semi-arid areas. In addition, Maymars is one of the most beautiful juniper species and is suitable for ornamental use (Piotto and Di Noi, 2003). Thus, information about the plant production of *Juniperus sabina* can be useful for forest managers and plant producers in many areas.

Berry extract of *Juniperus sabina* showed inhibitory activities against KB tumor cell lines (Sadeghi-aliabadi *et al.,* 2009). Fruit and leaves of junipers are commonly used as tea and pounded fruits are eaten to lower blood glucose levels in Anatolia. To evaluate antidiabetic and antioxidant potential and the chemical profile of *Juniperus sabina* L. in a study, phytochemical screening tests indicated the presence of flavonoids, tannins, terpenoids and carbohydrates in the extracts (Orhan *et al.,* 2017).

Maymars juniper is usually propagated by vegetative methods (Gheorghe *et al.,* 2010). To propagate plants via cuttings, the indole-butyric acid (IBA) growth regulator has been used as a treatment (Amri *et al.,* 2010). To produce junipers by stem cuttings, IBA has been used in previous studies (Henry *et al.,* 1992; Rifaki *et al.,* 2002). Research conducted by Rifaki *et al*. in 2002 on vegetative propagation showed the best concentration for the cuttings of junipers at 4000 ppm of IBA.

Phenolic compounds have effects on growth, development, propagation and plant defense (Croteau *et al.,* 2000). Measurement of internal compounds and their comparison during growth or rooting can be valuable factors in identifying internal barriers or enhancers of rooting in the cuttings, as there are no extensive resources available in this regard.

Phenolic compounds are a group of antioxidant agents (Choudhury *et al.,* 2013). Many scientists have reported the relationship between total phenol and antioxidant activities (Hariprasath *et al.,* 2015). In the propagation of varieties of blueberry, softwood cuttings and tissue culture, the interaction of genotype, propagation methods, and growth seasons significantly affected flavonoid content and antioxidant capacity. The interaction effect of the propagation method and genotype significantly affected total phenol and chlorophyll content. Also, the interaction between propagation method and growth season significantly affected the total flavonoid content (Goyali *et al.,* 2013).

Some studies have also revealed differences in rooting of cuttings as affected by substrate (Kentelky, 2011). Cocopeat and IBA were used to propagate *Juniperus excelsa* through stem cuttings, and they improved rooting ability (Esmael Nia *et al.,* 2006). Growth regulator and substrate are effective on the rooting of the cuttings of *Juniperus oblonga*, and proper substrate composition and the use of benzyl adenine increase the rooting of the cuttings (Khoshnevis *et al.,* 2012).

Roots uptake minerals and water from the soil (Chapin *et al.,* 1987). Higher numbers of adventitious roots could improve the root system's symmetry, stability, survival, and growth rate (Bryant and Trueman, 2015). Thus, rooting percentage is a good indicator of the growth strategies of root development and the capacity to endure water stress in Juniperus trees (Garcia Morote *et al.,* 2012).

Therefore, the present study is intended to investigate an efficient method of vegetative propagation of Maymars juniper using stem cutting and its effects on some of its phytochemical characteristics (phenolic compounds). We hypothesized that high level of phenolic compounds during rooting can be an indicator of the level of rooting in cuttings of *Juniperus sabina*, and that the percentage of rooting should be an indicator of rooting performance in cuttings. Thus, the objective of this research was to analyze the effects of five concentrations of IBA as treatment and four substrate types (perlite, perlite cocopeat, pumice, and mixed substrate) on the level of phenolic compounds and rooting performance in cuttings. The experiment was conducted in four months (February, mild climate; July, warm temperate climate; October, relatively cold weather; and January, cold weather) to determine the impact of harvesting time on the rooting capacity of cuttings.

2. Materials and Methods

Cutting preparation, treatment with indole butyric acid (IBA), and substrate composition.

The cuttings of *Juniperus sabina* were sampled from its natural habitat in the Chaharbagh mountains

of Gorgan, North Iran (Fig. 1), one of the main Mediterranean populations at higher altitude (2,700 m a.s.l.). Using a 30-year average, the mean annual temperature at the site is 9.2°C, and the mean annual precipitation is 429 mm. Extreme temperatures (summer and winter) range from 23°C to -5°C (data from Gorgan climatic station: 46° 06 N, 28° 00 W; 2,600 m a.s.l.). The crowns are approximately 2 x 2 m in length and width. The ring diameter of shrubs is 20.0 cm averagely, and the height is 1.5 m (these are old and horizontal shrubs). Generally, 20 male shrubs have been used for this experiment, and they are all growing in the same area with the same ecological environment. The experiment was conducted at Gorgan University of Agricultural Sciences and Natural Resources in winter, spring, summer, and fall of 2017. Stem cuttings were only collected from the upper crowns of male trees.

Fig. 1 - The worldwide distribution of different populations of *Juniperus sabina* (in grey) and the sampling area of stem cuttings (in a red circle) (Adams and Schwarzbach, 2016).

Cuttings were harvested in the morning. After harvesting, the stem cuttings were prepared to be 15 cm in length and 0.5-0.7 cm in diameter (Bohlenius *et al.,* 2017) for treating and cultivation in a greenhouse. Substrates were prepared, and cuttings were placed in the greenhouse equipped with an automatic system to control humidity (micro irrigation) and bottom heat. The average daily temperature during the experiment was 22°C, and the average relative humidity was 77%. The amount of light entering the greenhouse was varied based on the amount of natural light in each season.

For the treatment of stem cuttings, five levels of IBA were used: 0 or control, 1000, 2000, 4000, and 8000 mg L^{-1} (Control is a sample that is placed in the substrate without adding any treatment and is used to compare the effect of the treatments used on cuttings). The base of each cutting was placed in the aqueous solution of IBA for five seconds and then inserted into the substrate. The four used substrates were: I) perlite; II) mixed rooting substrate - a combination of sand (20%), perlite (20%), cocopeat (20%), vermicompost (20%), and potash (20%); III) perlite cocopeat (1:1), and IV) mineral pumice (each substrate about 10 Kg). For each treatment (combination of treatment and substrate), three replicates were prepared, with nine cuttings per replicate. Thus, a total of 540 cuttings in each season were cultivated.

Total phenol, flavonoids, and antioxidants of stem cuttings

Secondary metabolites were measured in both rooted and unrooted stem cuttings to detect differences in the internal compounds between cuttings that have the potential for rooting and others without this potential. For evaluating the treatments and to make comparisons between the chemical compounds in cuttings at the beginning of the sampling and the amount of increase or decrease between the time of planting and rooting (between the first and the end of each season), samples were taken from freshly harvested cuttings in each season (the first of each season with samples separately from the stem cuttings) and compared with the results at the end of the growing season.

In order to measure total phenol, antioxidants and flavonoids (at the end of each season and after harvesting the cuttings from substrate), in the first step, one gram of each plant sample, which was the bark of the stem of each cutting separately, was removed and powdered with liquid nitrogen, then placed in 10 cc of 80% methanol (Merk) in an Erlenmeyer flask, and after that, placed on a shaker for 24 h. The mixture was then filtered with filter paper and clean extracts were used to measure secondary metabolites in mg/g fresh weight (McDonald *et al.,* 2001). Then we began to assess the total phenolics, antioxidants, and flavonoids.

To measure total phenol, 20 µl of each of the above plant extracts were added to 1.16 µl of distilled water, 100 µl of folin (Merk) and 300 µl of sodium carbonate (20%), and they were mixed in a test tube (it is done for each plant sample separately) and then placed in a water bath at 45 °C for 30 min. After that, each sample was measured by a spectrophotometer (Unic-UV 2800 - 4 cells) at a wavelength of 760 nm. After drawing the standard graph (preparation of different concentrations with specific values of the control-different samples and readings with the spectrophotometer and then drawing on the curve) (Fig. 2), the phenol value of each sample was obtained (McDonald *et al.,* 2001).

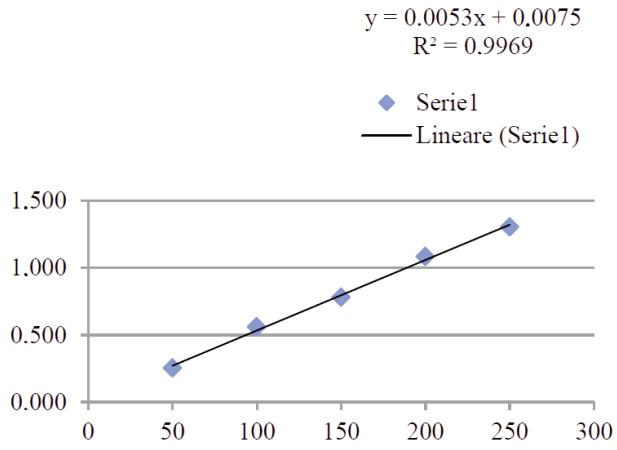

Fig. 2 - Standard graph of total phenol measurement.

To measure the flavonoids, 0.5 ml of each plant extract, 1.5 mg/L pure methanol (Merk), 0.1 ml of aluminum chloride, 0.1 ml of potassium acetate, and 2.8 ml of distilled water were combined and mixed in a test tube, and then all samples were placed in the dark for 30 minutes, and after that, they were measured by a spectrophotometer with a wavelength of 415 nm. After drawing the standard graph (preparation of different concentrations with specific values of the control-different samples with readings with the spectrophotometer and then drawing on the curve), the flavonoid value of each sample was obtained (Chong *et al.,* 2002) (Fig. 3).

To measure antioxidant activity, 1 ml of each plant extract was removed. In the next step, the amount of 0.0004 mg of DPPH was dissolved in 10 ml of methanol (Merk), and then 1 ml of this solution with 1 ml of each extract of the plant previously removed was combined, and finally, the antioxidant percentage was measured in a spectrophotometer with a wavelength of 517 nm (Miliauskas *et al.,* 2004).

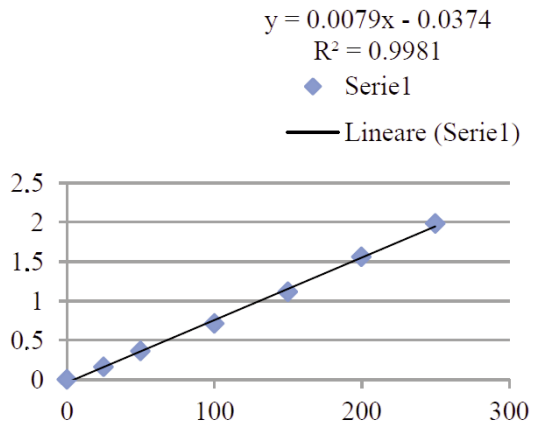

Fig. 3 - Standard graph of flavonoids measurement.

Rooting percentage

To determine the rooting percentage of each treatment, the roots were counted in all rooted cuttings (Fig. 4) in each treatment (three replications and each replication contained 9 cuttings; totally 27 cuttings) and then this number of cuttings was divided by 27 (some cuttings were unrooted and some of them were dried), (Negash, 2002).

Fig. 4 - Rooted cuttings.

Statistical analysis

A factorial arrangement of treatments (Hoshmand, 2006) was applied to analyze the effects of three main factors on five dependent variables. The first factor was “treatment” or concentration of IBA (five levels: 0, 1000, 2000, 4000, and 8000 ppm), the second was “substrate” (four levels: perlite, perlite-cocopeat, pumice, and mixed rooting substrate), and the third factor was “season” (four levels: January, April, July, and October). This represents a 5 x 4 x 4 factorial with 80 combinations of factor levels or treatments. The dependent variables were internal compounds of the cuttings (secondary metabolites in both unrooted and rooted cuttings) and the indicator of rooting performance (% of rooting). Therefore, in the dependent variables concerning chemical internal compounds, another level was added as treatment, secondary metabolites in fresh samples (stem cuttings not planted and prepared at the beginning of each season). This was done to compare the effects of treatments between cuttings not treated (at the beginning of each season) and treated cuttings at the end of each season.

SAS® statistical software (Neter *et al.,* 1996) was used to detect significant factors and to compare mean values between factors and levels of treatments. The comparison of the means was done using the PROC GLM procedure. We utilized Multifactor Analysis of Variance (a three-way ANOVA model) at a probability level of 5% ($p<0.05$). The analysis within season was performed by a two-way ANOVA (excluding season as a main factor in the complete model). In this research, we performed independent ANOVAs (not a mixed-design nor a repeated-measures ANOVA) because the measurements were indepen-

dent (we used different stem cuttings for each treatment and season).

A Fisher's Least Significant Difference (LSD) test ($p<0.05$) was used to determine the significant differences between treatments (Neter *et al.,* 1996). To apply this statistical method, it is desirable for data to be normally distributed. This is not the case with proportions, which have values that range between zero and one. In addition, errors must be independent and normally distributed with constant variance. To ensure these assumptions, a logarithmic transformation was used (Sabin and Stafford, 1990): for the percentage of rooting, the analyzed variable was [ln (r+0.5)], and r was the percentage of rooting (divided by 100). As this transformation requires numerical data above zero, a small number (0.5) was added to this variable before the transformation. The other dependent variables were normal and then distributed.

3. Results

Rooting performance

In Table 1, the p-values for the three principal effects (substrate, treatment with IBA and season, and their two-way interactions) and for the effects within each season (substrate, treatment, and their interactions) are represented. Effects must be considered significant when $p<0.05$. 540 stem cuttings in each season were planted. In January, five treatments rooted (99 cuttings), and 441 cuttings were unrooted. In April, 20 treatments rooted (502 cuttings) and 38 cuttings were unrooted. In July, four treatments rooted (89 cuttings) and 451 cuttings were unrooted. In October, four treatments rooted (102 cuttings) and 438 cuttings were unrooted.

As it is clear from figure 5, the best root-growing month is April. During spring, rooting was more than

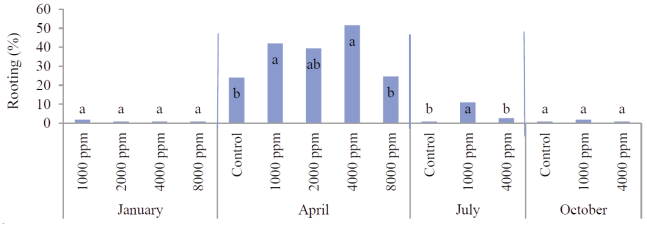

Fig. 5 - Mean values of rooting ability in rooted cuttings (percentage of rooting) within seasons and for the 5 treatments with indole butyric acid (IBA). The mean values for the same letter were not different at the 0.05 level according to the LSD test. Sample data = 540 cuttings for each month. For treatments not represented in the figure, all the cuttings dried. Error bars: LSD intervals.

50% at a level of 4000 ppm of indole butyric acid with no significant difference at the 1000 ppm level. Also, the minimum rooting percentage of the cuttings in this month was about 25% at the level of 8000 ppm of indole butyric acid and control treatment; however, it was higher than the rooting percentage of other months. In the study of the effect of different substrates on the percentage of rooting of the cuttings, the best substrate was seen in equal parts of perlite-cocopeat (v/v), with rooting at a maximum of 62% with a treatment of 1000 ppm (Fig. 6). And this substrate was one of the substrates that had the largest number and length of roots (Fig. 7 C and Fig. 8 C). Therefore, among the substrates used in this research to root the *Juniperus sabina*, the best substrate was perlite-cocopeat, with a maximum rooting percentage of 98. While the least rooting percentage of cuttings was seen in January, with less than 2% in all treatments, October is also not a good time for the reproduction of this plant. On the other hand, the most root number and root length was seen in April (Fig. 7 B and Fig. 8 B). So, the best months for rooting of cuttings of *Juniperus sabina* are April and May, and the best levels of IBA used were 4000 and 1000 ppm, Despite the fact that the largest number of roots was not seen in these treatments.

Table 1 - Results of the multifactor ANOVA to analyze the effects of the main factors on the rooting performance of cuttings across the four seasons

Variable	Effects	Growing season				Values
		January	April	July	October	
Rooting	Treatment	0.10	<0.001	0.0005	0.11	<0.0001
(log-transformed units)	Substrate	0.91	0.03	0.22	0.08	<0.0001
	Season	-	-	-	-	<0.0001
	Treatment x Substrate	-	0.24	-	-	<0.0001
	Treatment x Season	-	-	-	-	<0.0001
	Substrate x Season	-	-	-	-	<0.0001

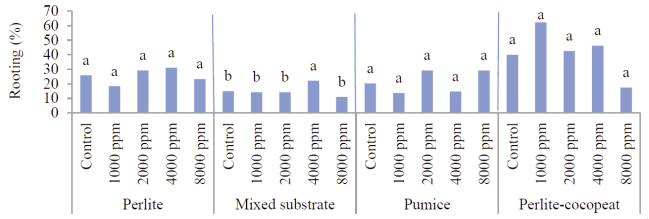

Fig. 6 - The mean values of rooting performance in rooted cuttings (percentage of rooting) within substrates and for the 5 treatments of indole butyric acid. The mean values with the same letter were not different at level 0.05 according to the LSD test. Sample data: 540 cuttings for each substrate.

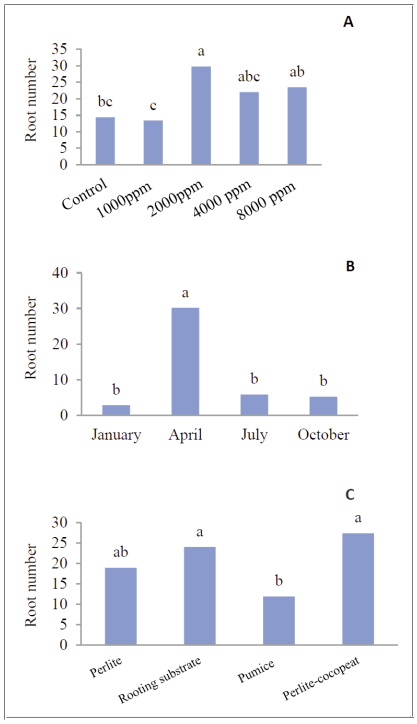

Fig. 7 - A, B, C The mean values of rooting performance in rooted cuttings (root number) within for the 5 treatments of indole butyric acid, 4 season and 4 substrates. The mean values with the same letter were not different at level 0.05 according to the LSD test. Sample data: 540 cuttings for each substrate.

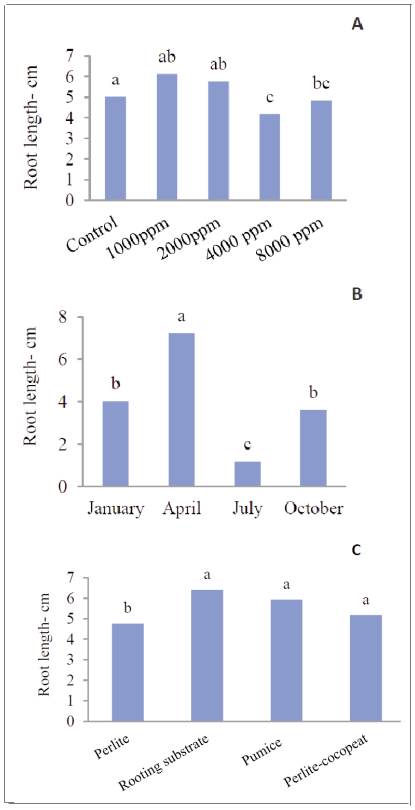

Fig. 8 - A, B, C The mean values of rooting performance in rooted cuttings (root length) within for the 5 treatments of indole butyric acid, 4 season and 4 substrates. The mean values with the same letter were not different at level 0.05 according to the LSD test. Sample data: 540 cuttings for each substrate.

Secondary metabolites concentration

The results of the main and interaction effects of different treatments are presented in Table 2. Based on the results, each of the measured factors has been interpreted and reviewed.

Phenol content

As it is shown in figure 9 A, among treatments in unrooted cuttings, the highest total phenol content

Table 2 - Results of a multifactor ANOVA used to examine the effect of major factors on the secondary metabolite composition of stem cuttings over four seasons

Factors	DF	Phenol		Flavonoid		Anti-Oxidant	
		Unrooted Cuttings	Rooted Cuttings	Unrooted Cuttings	Rooted Cuttings	Unrooted	Rooted Cuttings
IBA	4	16675.96 *	54417.29 **	19.35.41 ns	2034.93 NS	249.75 NS	813.69 **
Season	3	161064.55 **	19288.21 NS	84936.79 **	12674.40 **	10155.84 **	11007.76 **
Substrate	3	24699.73 **	14171.04 NS	2281.75 ns	1549.77 NS	337.35 NS	124.01 NS
IBA x Season	6	55862.72 **	93512.97 **	9142.79 **	9016.74 **	840.18 **	1411.13 **
IBA x Substrate	12	9947.75 NS	4875.10 NS	1489.16 NS	794.45 NS	118.72 NS	215.88 *
Season x Substrate	3	10173.14 NS	263.48 NS	2666.17 NS	0.00 NS	139.36 NS	6.48 NS
IBA x Season x	0	7509.96 NS	2371.40 NS	1690.41 NS	-	163.35 NS	38.92 NS
Error	66	5661.43	8792.53	1604.33	1818.08	170.08	90.61

In the table, the p-values for the three principal effects (substrate, treatment with IBA and season, and their two-way interactions) are represented. Effects were considered significant when p<0.05. 540 stem cuttings in each season were planted.* p<0.5.

(McDonald *et al.,* 2001) was observed with no significant difference in the fresh sample as well as in 4000 ppm and 8000 ppm of indole butyric acid treatments, and the lowest level was observed in control, 1000 ppm, and 2000 ppm treatments without any significant difference. A fresh sample was prepared with other cuttings at the beginning of each season and is used only to measure the internal composition of the plant at the beginning of the season; no treatment is performed on it. It was to compare the amount of internal compounds of the plant at the beginning of the cutting time and compare it with the amount of these compounds after maintaining the cuttings in the substrate to root (control is a sample that is placed in the substrate without adding any treatment and is used to compare the effect of the treatments used on cuttings).

Among the different substrates, the lowest amount of phenol content was found in stem cuttings that were planted in the mixed rooting substrate (Fig. 9 B). Among the unrooted cuttings, the lowest phenol content was observed in a fresh sample and a treatment of 1000 ppm in January (Fig. 9 C). Between rooted cuttings, in April, with the highest rooting percentage of cuttings, treatments of 4000 and 8000 ppm showed lower phenol content, and there was no significant difference between other treatments (Fig. 9 D).

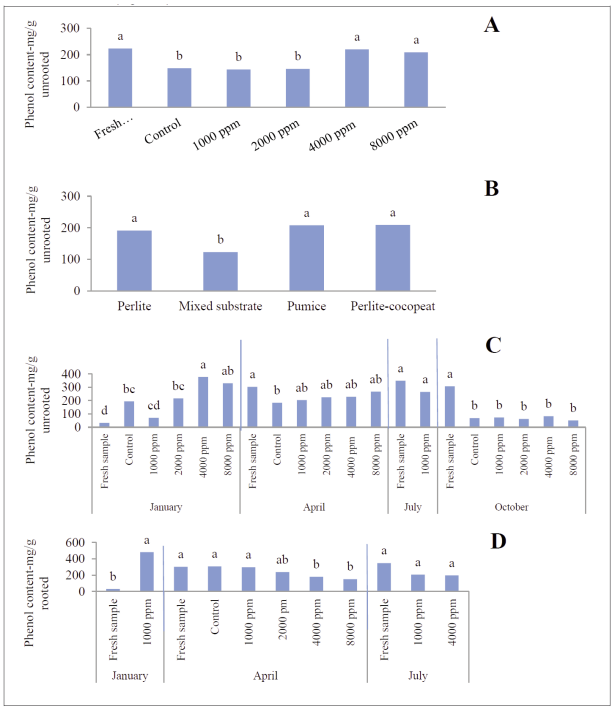

Fig. 9 - A, B, C, D Mean internal phenol content within seasons for the different treatments with IBA for rooted and unrooted cuttings (a, b, c, d). The mean values for the same letter were not significantly different at the 0.05 level according to the LSD test. 540 stem cuttings in each season were planted. For the treatments that were not represented in the figure, those cuttings have dried.

Flavonoid content

Among the unrooted cuttings in different seasons, the highest flavonoid levels were observed in January and July, and the lowest were seen in April and October (Fig. 10 A). The flavonoid content of *Juniperus sabina* differed during different seasons.

In rooted cuttings, flavonoid content was not significantly different in treatments applied in different months, and the overall amount of flavonoid was between 50 and 100 mg/g of fresh weight (Fig. 10 B).

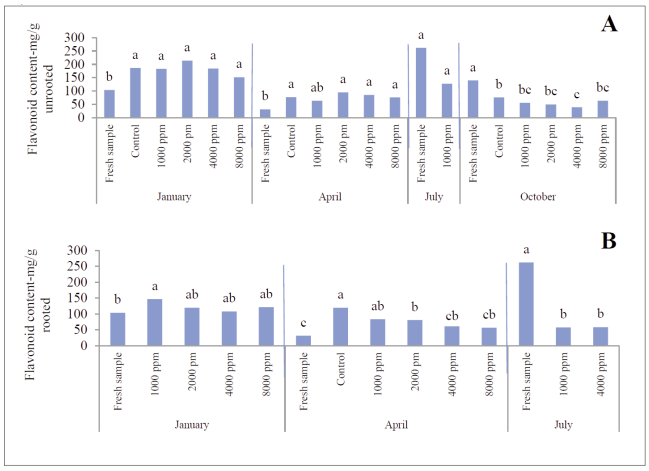

Fig. 10 - A, B. The mean internal flavonoid content within seasons for the different treatments with IBA for rooted and unrooted cuttings. The mean values for the same letter were not significantly different at the 0.05 level according to the LSD test. 540 stem cuttings in each season were planted. For treatments not represented in the figure, all the cuttings dried.

Percentage of antioxidant

In unrooted cuttings, the highest percentage of antioxidants was found in January and October with more than 70%, and the lowest was observed in July with a maximum of 20%. In April, an intermediate level of antioxidants was observed in unrooted cuttings (Fig. 11 A). In all months except July, the percentage of antioxidants in the first samples was about 70%, but in July it was about 20%. It should be noted that in July and January, the percentage of antioxidants increased after treating and planting the cuttings in substrate; this amount was unchanged in October (during fall) and it decreased in April (during spring), and its decline was also significant.

In rooted cuttings, the percentage of antioxidants in January was much higher April (Fig. 11 B). Among

different substrates, the lowest percentage of antioxidants in rooted cuttings was seen in mixed rooting substrate, and its maximum was seen in perlite substrate (Fig. 11 C). Pumice and perlite-cocopeat substrate showed a medium antioxidant percentage. It means in the lighter substrate, the antioxidant percentage was increased, and in the heavier substrate, the percentage of that was decreased.

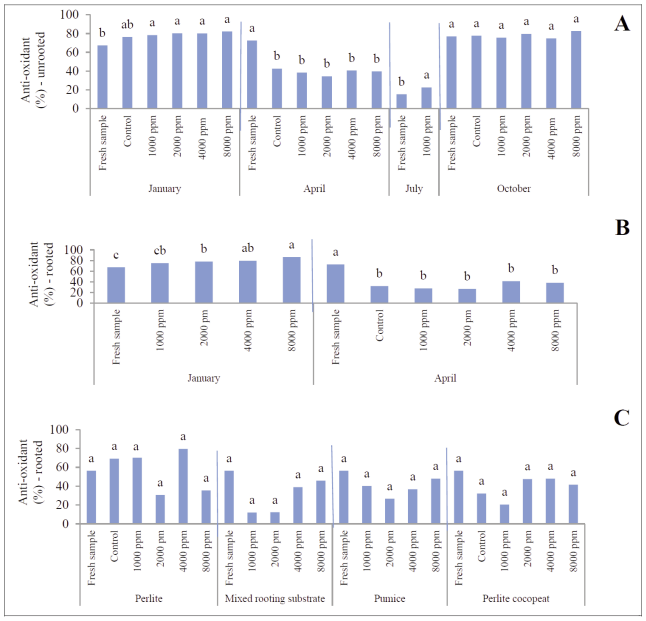

Fig. 11 - A, B, C. The mean internal antioxidant percentage within seasons for the different treatments with IBA for rooted and unrooted cuttings. Mean values for the same letter were not significantly different at the 0.05 level according to the LSD test. 540 stem cuttings in each season were planted. For treatments not represented in the figure, all the cuttings dried.

4. Discussion and Conclusions

Indole butyric acid is widely used at commercial level to root many species (Hartmann *et al.,* 1990; Negash, 2002; Esmael Nia *et al.,* 2006; Khoshnevis *et al.,* 2012). It slowly releases a source of indole acetic acid (Epstein and Ludwig-Muller, 1993). Current evidence indicates that indole butyric acid is naturally occurring in plants. Further stability of IBA in comparison with indole acetic acid during rooting experiments has been reported by Nordstrom *et al.* (1991), which is effective on decomposition and building. Part of the function of indole butyric acid is the direct effect of auxin (Ludwig-Muller, 2000; Poupart and Waddell, 2000). Although other functions are due to the conversion of IBA to IAA by b-oxidation (Epstein and Lavee, 1984; Zolman *et al.,* 2000; Bartel *et al.,* 2001).

Auxin can be increased for up to 24 hours after sampling (Tartoura *et al.,* 2004; Osterc *et al.,* 2009). Increasing root numbers after the use of indole butyric acid occurs in many woody plants (Jarvis, 1986). Adventitious roots on the cuttings were created by treating them with auxin growth regulators, especially indole butyric acid (Buchala and Schmid, 1979; Haissig *et al.,* 1992). This is consistent with the results of this research on its effectiveness on rooting. One possible explanation is that exogenous auxins can increase the amount of internal auxin in the direction of the onset of the formation phase of the rooting and then the root appearance (Metaxas *et al.,* 2004). With an increase in the presence of the cuttings in the substrate, the rooting rate of the cuttings also increases (Cope and Rupp, 2013). The use of indole butyric acid leads to an increase in rooting (Bielenin, 2003).

In our study, the best results were obtained from intermediate levels of IBA (1000-4000 ppm) without a significant difference between these treatments, and we hypothesize that IBA at 8000 ppm can damage the cuttings and reduce rooting. In *J. virginiana*, IBA concentrations up to 2000 ppm did not stimulate rooting beyond that obtained with 5000 ppm (Henry *et al.,* 1992). In general, IBA has been used for the rooting of *Juniperus* species with different treatment levels. For example, results were best at 8000 ppm of IBA in *Juniperus osteosperma* (Cope and Rupp, 2013), 5000 ppm of IBA in *J. virginiana* (Henry *et al.,* 1992), 1000 ppm to 9000 ppm in *Juniperus scopulorum* (Bielenin, 2003), Chowdhuri (2017), with 1000 to 3000 ppm in *Juniperus chinensis* and Tektas *et al.,* (2017) with 6000 ppm in Juniperus L. In the research on *Juniperus virginiana*, Henry *et al.* (1992) cited that in preliminary studies, IBA concentrations up to 20000 ppm did not stimulate rooting beyond that obtained with 5000 ppm. Thus, our results are more in agreement with Rifaki *et al.* (2002), which proposed a concentration of 4000 ppm of IBA in cuttings of *Juniperus excelsa*, and Esmaeil Nia *et al.* (2006), with 3000 to 6000 ppm in *J. excelsa*. Nevertheless, the novelty of our results is that the concentration of IBA we selected (1000 ppm) was lower.

Substrate characteristics are very important in rooting success. Several studies have shown that the substrate plays a significant role in the quality of root formation and the percentage of rooted cuttings. Proper air preservation is a necessary feature of a

good rooting atmosphere. Therefore, it seems that proper rooting substrate can maintain proper moisture to prevent the cutting ends from drying out and to provide enough air to facilitate rooting and prevent disease spread at the base of the cuttings. Surely there is an optimum temperature for substrate for root formation and growth, and rooting at low temperatures will not occur or will occur very slowly. It is also possible for the roots to appear and grow at very high temperatures in the substrate. Bottom-heat is useful for rooting only when the temperature is low (Couvillon, 1988) which is consistent with the results of this research. In our study, the percentage of rooting was more than 60% in substrate of perlite cocopeat. In an study of *Juniperus procumbens* the best substrate was 1.3 (v/v) vermiculite and 2.3 (v/v) perlite, with only 36% rooting (Hong-wei *et al.,* 2011). The results of our study was also better than the results obtained from Cuevas-Cruz *et al.* (2015) in Pinus, with 43.5% of rooting (substrate was a mixture of peat-perlite-vermiculite), Khoushnevis *et al.* (2012) with 28% of rooting in *Juniperus oblonga* (fine and harsh bed), Stuepp *et al.* (2014), with 16% of rooting in *J. chinensis* (fine grained vermiculite and carbonized rice hull 1:1) and Ayan *et al.* (2004), with 24% of rooting for *J. foetidissima*, 31.5% of rooting for *J. excelsa*, 38.42% of rooting for *Juniperus sabina* and 31.83% of rooting for *J. oxycedrus* (perlite).

Cutting time plays an important role in the success of rooting. Although many species are most rooted when cuttings are prepared in late spring or early winter before the wood has hardened, many other species have the best rooting when cuttings are taken at other times of the year. A good example of this is the *Juniperus horizontalis*, whose cuttings were most rooted when they were prepared between November and February compared to other times of the year (Ali Ahmad Koruri *et al.,* 2011). The result of this research showed that the best time for rooting *Juniperus sabina* to prepare the cuttings is April. Therefore, for this species, the best time to prepare cuttings and plant them is spring. This differs from Guerrero-Campo *et al.* (2006), who found the best rooting of several species of cuttings at different seasons and Chowdhuri (2017), who showed the best rooting time for *Juniperus chinensis* was summer. On the other hand, our result was in agreement with Fragoso *et al.* (2015) and Tektas *et al.* (2017), who respectively cited the best season for rooting of *Juniperus chinensis* and Juniperus L. as spring.

Apparently, the presence of secondary metabolites in plants acts as a defense (toxic) agent that inhibits proliferation and other growth-related actions (Singh Rattan, 2010), as shown in the results of this study. Although most of the phenolic compounds have a structural role in the cell wall, the major activity of these compounds is in defense of the plant; they have several roles in plants, but are mainly used for their great effects on growth, development, propagation, as well as plant defense against animals and pathogens (Croteau *et al.,* 2000). The presence and yield of secondary metabolites in plants, such as aromatic compounds and compounds in essential oils, may be affected in different ways, from formation to separation from plants. Rapid secondary metabolite induction occurs as a chemical mediator of plant rooting and defense (Metlen *et al.,* 2009), and the amount of secondary metabolites changed during the preservation of cuttings in the substrate. The rooting barrier of yew cuttings was identified by biological and organic methods. The results showed that the most important barrier to propagation in this plant was phenol content (Guangyou, 2000).

The maximum amount of total phenol in the leaves of common juniper was 315.33 mg/g (Ved *et al.,* 2017), which is consistent with the results of this study. In cherry leaf cuttings, GiSelA 5, auxin had no effect on phenol levels, so the same results were observed in the present study. Cuttings should have definite levels of different phenolic compounds to start the rooting induction phase, but the greater effect on rooting success is attributed to the effect of auxin level (Trobec *et al.,* 2004).

Phenolic compounds are a class of antioxidants (Choudhury *et al.,* 2013), and the level of internal antioxidants in plants is different (Rehman *et al.,* 2014). Many authors have reported an association between total phenol content and antioxidant activity (Hariprasath *et al.,* 2015). The main antioxidant activity is due to specific secondary metabolites, especially phenolic compounds and some terpenes (Marzouk *et al.,* 2007; Awaad and Al-Jaber, 2010).

Interactions among genotypes, propagation methods, and growing seasons significantly affect flavonoid content and antioxidant capacity (Goyali *et al.,* 2013), which is consistent with the results of this study. The amount of secondary compounds varied according to season and substrate, just as it did in the current study. The climate of the outdoor region during the three months of October, January, and

April increased the amount of antioxidants inside the plant, while in July, with a hot climate, it dropped dramatically. Growth regulators increase antioxidant activity (Dakah *et al.,* 2013), which contradicts the results of this research. Because in some cuttings treated with indole butyric acid, an increase in antioxidants was observed, and in other treatments, a decrease was observed.

The results of a study on one of the Iranian conifers showed that the antioxidant activity of the extracts ranged between 60 and 99% (Hariprasath *et al.,* 2015), which contradicts the result of the present study, which shows that the range of antioxidants in some treatments was less than 20%.

In the use of indole butyric acid for the propagation of *Juniperus sabina* through cuttings, the best rooting month (season) for cuttings was April, and the rooting percentage in this month was higher than in other months (more than 50%), while instead, the lowest rooting rate was seen in January. The best levels of indole butyric acid used were levels of 4000 and 1000 ppm, respectively. So, for the propagation of Sabina species, it is recommended to use these levels of IBA as a treatment for stem cuttings in April. Also, the best substrate used was perlite-cocopeat. Between rooted cuttings, in April, with the highest rooting percentage of cuttings, treatments of 4000 and 8000 ppm showed lower phenol content; flavonoid content was not significantly different in treatments applied in different months and the percentage of antioxidants in January was much higher than April.

References

ADAMS R.P., SCHWARZBACH A.E., 2016 - *Chloroplast capture by a new variety,* Juniperus sabina *var.* Balkanensis R. P. Adams *and* A. N. Tashev, *from the* balkan peninsula: *A putative stabilized relictual hybrid between* Juniperus sabina *and ancestral* J. thurifera. - Phytologia, 98: 100-111.

ALI AHMAD KORURI S., KHOUSHNEVIS M., MATINIZADEH M., 2011 - *Comprehensive studies of juniper species in Iran*. - First ed, Pooneh, Tehran, Iran, pp. 550.

AMRI E., LYARUU H., NYOMORA A., KANYEKA Z., 2010 - *Vegetative propagation of african blackwood* (Dalbergia melanoxylon *Guill. & Perr.*): *Effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings.* - New Forests, 39: 183-194.

AWAAD S.A., AL-JABER A.N., 2010 - *Antioxidant natural plant, Ethnomedicine: Source and mechanism*. - Ethnomedicine: Source & Mechanism I, 27: 135.

AYAN S., KÜÇÜK M., ULU F., GERÇEK V., SAHIN A., SIVACIOĞLU A., 2004 -*Vegetative Propagation Possibilities of Some Natural Juniper* (Juniperus L.) *Species*. - J. Forestry Faculty, 4(1): 1-12.

BARTEL B., LECLERE S., MAGIDIN M., ZOLMAN B.K., 2001 - *Inputs to the active indole-3-acetic acid pool: de novosynthesis, conjugate hydrolysis, and indole-3-butyric acid b-oxidation*. - J. Plant Growth Reg., 20: 198-216.

BIELENIN M., 2003 - *Rooting and gas exchange of* conifer *cuttings treated with indolebutyric acid.* - J. Fruit Ornam. Plant Res., 11: 99-105.

BOHLENIUS H., FRANSSON T., HOLMSTROM E., SALK C., 2017 - *Influence of cutting type and fertilization in production of containerized poplar plants*. - Forests, 8(5): 164.

BRYANT P., TRUEMAN S., 2015 - *Stem anatomy and adventitious root formation in cuttings of* Angophora, Corymbia and Eucalyptus. - Forests, 6: 1227-1238.

BUCHALA A.J., SCHMID A., 1979 - *Vitamin D and its analogues as a new class of plant growth substances affecting rhizogenesis*. - Nature, 280: 230-231.

CHAPIN F., BLOOM A., FIELD C., WARING R., 1987 - *Plant-responses to multiple environmental factors*. - Bioscience, 37: 49-57.

CHONG C., YANG M., WEN H., CHERN J., 2002 - *Estimation of total flavonoid content in propolis by two complementary colorimetric methods*. - J. Food Drug Analysis, 10: 178- 182.

CHOUDHURY D., GHOSAL M., DAS A., MANDAL P., 2013 - *Development of single node cutting propagation techniques and evaluation of antioxidant activity of* curcuma aeruginosa *roxburgh rhizome*. - Int. J. Pharmacy Pharmac. Sci., 5 (2): 227-234.

CHOWDHURI T.K., 2017 - *Performance evaluation of different growth regulators on propagation of Chinese juniper* (Juniperus chinensis *Var. pyramidalis*) *in subtropical zone*. - J. Pharmacogn. Phytochem., 6(5): 2190-2193.

COPE K., RUPP L., 2013 - *Cutting Propagation of* Juniperus osteosperma (*Utah Juniper*). - Native Plants J., 14(2): 76-84.

COUVILLON C., 1988 - *Rooting responses to different treatmens*. - Acta Horticulturae, 227: 187-196.

CROTEAU R., KUTCHAN M.T., LEWIS G.N., 2000 - *Natural Products (Secondary Metabolites)*. - Amer. Soc. Plant Physiologists, USA, pp. 1250-1318.

CUEVAS-CRUZ J., JIMENEZ-CASAS M., JASSO-MATA J., PEREZ-RODRIGUEZ P, LOPEZ-UPTON J, VILLEGAS-MONTER A., 2015 - *Asexual propagation of* Pinus leiophylla schiede *ex schltdl. Et cham*. - Revista Chapingo Serie Ciencias Forestales Y Del Ambiente, 21: 81-95.

DAKAH A., ZAID S., SULEIMAN M., ABBAS S., WINK M., 2013 - In vitro *micropropagation of the medicinal plant* Ziziphora tenuior L. *and evaluation of its antioxidant*

activity. - Saudi J. Biol. Sci., 62 (13): 114-119.

EPSTEIN E., LAVEE S., 1984 - *Conversion of indole-3-butyric acid to indole-3-acetic acid by cuttings of grapevine* (Vitis vinifera) *and olive* (Olea europea). - Plant Cell Physiology, 25: 697-703.

EPSTEIN E., LUDWIG-MULLER J., 1993 - *Indole-3-butyric acid in plants: occurrence, biosynthesis, metabolism, and transport*. - Physiologia Plantarum, 88: 382-389.

ESMAEL NIA M., JALALI S.GH., TABARI M., HOSSEINI S.M., 2006 - *Influence of plant growth regulator IBA on vegetative propagation of* Juniperus excels. - Iran. J. For. Pop. Res., 14: 221-227.

FRAGOSO R., ZUFFELLATO-RIBAS K. CH., MACANHÃO G., STUEPP C. A., KOEHLER H. S., 2015 - *Vegetative propagation of* Juniperus chinensis. - Comunicata Scientiae, 6(3): 307-316.

GARCIA MOROTE F.A., LOPEZ SERRANO F.R., ANDRES M., RUBIO E., GONZALEZ JIMENEZ J.L., DE LAS HERAS J., 2012 - *Allometries, biomass stocks and biomass allocation in the thermophilic Spanish juniper woodlands of southern Spain*. - Forest Ecol. Manag., 270: 85-93.

GHEORGHE B., ILEANS A., CORNELIA P., 2010 - *Research regarding the influence of some growth biostimulating substances on the rooting of* Juniperus horizontalis *seedlings*. - Analele Universitatii din Oradea, Fascicula: Protectia Mediului, 15: 23-27.

GOYALI J., IGAMBERDIEV A., DEBNATH S., 2013 - *Morphology, phenolic content and antioxidant capacity of lowbush blueberry* (Vaccinium angustifolium *Ait.) plants as affected by* in vitro *and* ex vitro *propagation methods.* - Canadian J. Plant Sci., 93(6): 1001-1008.

GUANGYOU C., 2000 - *Determination of rooting-inhibitor in cutting of* Taxus cuspidate. - J. Beihua University (Natural Science), 1(2): 163-166.

GUERRERO-CAMPO J., PALACIO S., PEREZ-RONTOME C., MONTSERRAT-MARTI G., 2006 - *Effect of root system morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in north-east spain*. - Annals Bot., 98: 439-447.

HAISSIG B.E., DAVIS T.D., RIEMENSCHNEIDER D.E., 1992 - *Researching the controls of adventitious rooting*. - Physiol. Plant., 84: 310-317.

HARIPRASATH L., JEGADEESH R., ARJUN P., RAAMAN N., 2015 - In vitro *propagation of* Senecio candicans *DC and comparative antioxidant properties of aqueous extracts of the* in vivo *plant and* in vitro-*derived callus.* - South African J. Bot., 98: 134-141.

HARTMANN H.T., KESTER D.E., DAVIES F.T., 1990 - *Plant propagation: principles and practices*. - Prentice Hall, New Jersey, pp. 246-247.

HENRY P.H., BLAZICH F.A., HINESLEY L.E., 1992 - *Vegetative propagation of eastern red cedar by stem cuttings.* - HortSci., 27: 1272-1274.

HONG-WEI Y., YONG-SHENG G., HAI-JUN S., YAN-HE R., 2011 - *Experiment on cutting propagation of* sabina procumbens. - Journal of Inner Mongolia Forestry Science and Technology, Abstract.

HOSHMAND H.R., 2006 - *Design of experiments for agriculture and the natural sciences second edition*. - Second Edition, New York, USA, pp. 437.

JARVIS B.C., 1986 - *Endogenous control of adventitious rooting in nonwoody cuttings*, pp. 191-222. - In: JACKSON M.B. (ed.) *New root formation in plants and cuttings*. Martinus Nijhoff, UK, pp. 282.

KENTELKY E., 2011 - *The analysis of rooting and growth peculiarities of Juniperus species propagated by cuttings.* - Horticulture, 68: 380-385.

KHOSHNEVIS M., TEIMOURI M., MATINIZADEH M., SHIRVANY A., 2012 - *Effect of hormones, light and media treatments on rooting of* juniperus oblonga *cuttings*. - Iranian J. Forest, 4(2): 135-142.

LUDWIG-MULLER J., 2000 - *Indole-3-butyric acid in plant growth and development*. - Plant Growth Regul., 32: 219-230.

MARZOUK M., MOHARRAM F., MOHAMED M., GAMALELDEEN A., ABOUTABL E., 2007 - *Anticancer and antioxidant tannins from* Pimenta dioica *leaves*. - Z. Naturforsch, 62: 26-36.

MCDONALD S., PRENZLER P. D., AUTOLOVICH M., ROBARDS K., 2001 - *Phenolic content and antioxidant activity of olive extract*. - Food Chem., 73: 73- 48.

METAXAS D., SYROS T., YUPSANIS T., ECONOMOU A., 2004 - *Peroxidases during adventitious rooting in cuttings of* Arbutus unedo *and* Taxus baccata *as affected by plant genotype and growth regulator treatment*. - Plant Growth Regul., 44: 257-266.

METLEN L.K., ASCHEHOUG T. E., CALLAWAY M.R., 2009 - *Plant behavioral ecology: dynamic plasticity in secondary metabolites*. - Plant Cell Envir., 32: 641-653.

MILIAUSKAS G., VENSKUTONIS P.R., VAN BEEK T. A., 2004 - *Screening of radical scavenging activity of some medicinal plants and aromatic plant extract*. - Food Chem., 85: 231-237.

NEGASH L., 2002 - *Successful vegetative propagation techniques for the threatened African pencil cedar* (Juniperus procera *Hoechst. Ex Endl*.). - Forest Ecology Management, 161: 53-64.

NETER J., KUTNER M., WASSERMAN W., NACHTSHEIM C., 1996 - *Applied linear statistical models*. - 4th ed, McGraw-Hill-Irwin, Chicago, USA, pp. 720.

NORDSTROM A.C., ALVARADO JACOBS F., ELIASSON L., 1991 - *Effect of exogenous indole-3-acetic acid and indole-3- butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings*. - Plant Physiol, 96: 856-861.

ORHAN N., DELIORMAN ORHAN D., GÖKBULUT A., ASLAN M., ERGUN F., 2017 - *Comparative analysis of chemical profile, antioxidant,* in-vitro *and* in-vivo *antidiabetic activities of* Juniperus foetidissima *Willd. and* Juniperus sabina *L*. - Iran J. Pharm. Res., 16: 64-74.

OSTERC G., TEFANCIC M.S., TAMPAR F.S., 2009 - *Juvenile*

stockplant material enhances root development through higher endogenous auxin level. - Acta Physiol. Plant., 31: 899-903.

PIOTTO B., DI NOI A., 2003 - *Seed propagation of Mediterranean trees and shrubs*. - Agency for the Protection of the Environment and for Technical Services, Roma, Italy, pp. 108.

POUPART J., WADDELL C.S., 2000 - *The rib1 mutant is resistant to indole-3-butyric acid, an endogenous auxin in Arabidopsis*. - Plant Physiol., 124: 1739-1751.

REHMAN R.U., CHAUDHARY M.F., KHAWAR K.M., LU G., MANNAN A., ZIA M., 2014 - In vitro *propagation of* Caralluma tuberculata *and evaluation of antioxidant potential*. - Biologia, 69(3): 341-349.

RIFAKI N., ECONOMOU A., HATZILAZAROU S., 2002 - *Factores affecting vegetative propagation of* Juniperus excels *Bieb by stem cutting*. - Prop. Ornam. Plants, 2(2): 9-13.

SABIN T.E., STAFFORD S.C., 1990 - *Assessing the need for transformation of response variables*. - Forestry Publications Office, Oregon State University, Corvallis, USA, pp. 38.

SADEGHI-ALIABADIA H., EMAMIB A., SAIDIA M., SADEGHIA B., JAFARIANC A., 2009 - *Evaluation of* in vitro *cytotoxic effects of* Juniperus foetidissima *and* Juniperus sabina *extracts against a panel of cancer cells.* - Iranian J. Pharm. Res., 8(4): 281-286.

SINGH RATTAN R., 2010 - *Mechanism of action of insecticidal secondary metabolites of plant origin*. - Crop Protection, 29: 913-920.

STUEPP C.A., RUFFELLATO-RIBAS K.C., MACANHÃO G., FRAGOSO R., RICKLI H.C., 2014 - *Rooting of* Juniperus chinensis *var. Kaizuka for different concentrations of iba and heights collection*. - Revista Agrarian, 7: 496-503.

TARTOURA K., DA ROCHA A., YOUSSEF S., 2004 - *Synergistic interaction between coumarin 1, 2-benzopyrone and indole-3-butyric acid in stimulating adventitious root formation in* Vigna radiata *(L.) Wilczek cuttings: I. Endogenous free and conjugated IAA and basic isoperoxidases*. - Plant Growth Regul., 42: 253-262.

TEKTAS I., TÜRKOĞLU N., CAVUSOĞLU S., 2017 - *Effects of auxin doses on rooting of* Juniperus L. - Progress in Nutrition, 19 (1): 130-136.

TROBEC M., STAMPAR F., VEBERIC R., OSTERC G., 2004 - *Fluctuations of different endogenous phenolic, compounds and cinnamic acid in the first days of the rooting process of cherry rootstock 'GiSelA 5' leafy cuttings*. - J. Plant Physiol., 162: 589-597.

VED A., GUPTA A., KUMAR SINGH RAWAT A., 2017 - *Antioxidant and Hepatoprotective potential of phenol-rich fraction of* Juniperus communis *Linn. leaves*. - Pharmacogn. Mag., 13(49): 108-113.

WESTERFIELD R.R., 2012 - *Junipers*. - Trafford, USA, pp. 1-8.

ZOLMAN B.K., YODER A., BARTEL B., 2000 - *Genetic analysis of indole-3-butyric acid response in* Arabidopsis thaliana *reveals four mutant classes*. - Genetics, 156: 1323-1337.